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The role of endophytic methane oxidizing bacteria in submerged *Sphagnum* in determining methane emissions of Northeastern Siberian tundra

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Abstract

The behavior of tundra ecosystems is critical in the global carbon cycle due to their wet soils and large stores of carbon. Recently, cooperation was observed between methanotrophic bacteria and submerged *Sphagnum*, which reduces methane emissions in this type of vegetation and supplies CO_2 for photosynthesis to the plant. Although proven in the lab, the differences that exist in methane emissions from inundated vegetation types with or without *Sphagnum* have not been linked to these bacteria before.

To further investigate the importance of these bacteria, chamber flux measurements, microbial analysis and flux modeling were used to show that methane emissions in a submerged *Sphagnum*/sedge vegetation type were 50% lower compared to an inundated sedge vegetation without *Sphagnum*. From examining the results of the measurements, incubation experiments and flux modeling, it was found that it is likely that this difference is due to, for a large part, oxidation of methane below the water table by these endophytic bacteria.

This result is important when upscaled spatially since oxidation by these bacteria plays a large role in 15% of the net methane emissions, while at the same time they promote photosynthesis of *Sphagnum*, and thus carbon storage. Future changes in the spread of submerged *Sphagnum*, in combination with the response of these bacteria to a warmer climate, could be an important factor in predicting future greenhouse gas exchange from tundra.

1 Introduction

Tundra currently covers an area of around 8.8×10^6 km^2 globally. Over thousands of years, wet soil conditions, in combination with a cold climate, have led to the buildup of large deposits of carbon in tundras at northern latitudes. These are estimated to be as large as ±200 Pg (Post et al., 1982), although it has recently been suggested that this may be a substantial underestimate (Tarnocai et al., 2009). Changes in the magnitude

BGD
7, 8521–8551, 2010

of carbon fluxes from this area are thus very important to the global carbon cycle. The tundra ecosystem has come under increasing pressure since air temperatures have risen much faster in the arctic than in the rest of the world (Serreze et al., 2000; Johannessen et al., 2004), and this increase is expected to continue (Zwiers, 2002; Chapin III et al., 2005). An improved understanding of the biogeochemical functioning of tundras is thus urgently needed to better appreciate its response to these changes.

While tundra generally is a sink for carbon, it can be a source of methane when the soil is wet (Corradi et al., 2005; van der Molen et al., 2007; Wille et al., 2008). Cumulatively, methane is the second most important greenhouse gas (Frolking et al., 2006). It is formed by archaea in the anoxic part of the soil as the final step in the series of processes that degrade organic matter (Whalen, 2005). The gas thus produced can either be recycled in the soil by other microorganisms and oxidized to CO_2 or emitted to the atmosphere.

Since methane production requires anoxic conditions, emissions are sensitive to the position of the water table. In the aerated part of the soil, above the water table, methanotrophic bacteria convert methane to CO_2 and if the aerated part of the soil is deep enough, most or all of the methane can be consumed (Whalen and Reeburgh, 1990). Methane emissions are therefore at their highest when the water table is situated close to or at the surface. In tundra, these areas tend to be dominated by sedges such as *Eriophorum* spp., *Carex* spp. and mosses such as *Sphagnum* spp.

Importantly, emissions of methane are also influenced by the presence of vascular plants. They facilitate a release of methane through their aerenchyma that provide a direct pathway to the atmosphere, bypassing oxic zones in the soil where methane would normally be oxidized (Joabsson et al., 1999; Greenup et al., 2000; Christensen et al., 2003; Ström et al., 2005). However, the very same plant structure also increases the transfer of oxygen into the soil, which can lead to significant oxidation of methane at the rhizosphere (Popp et al., 2000; Whalen, 2005), although there have been studies that have shown that little or no oxidation can occur in the sedges *Eriophorum angustifolium* and *Eriophorum vaginatum* (Frenzel and Rudolph, 1998). Furthermore, vegetation also

BGD
7, 8521–8551, 2010

influences the quality of substrate that is available for carbohydrate oxidation. Methane production is higher in the presence of more labile carbon than with dominantly stable organic matter and it has been shown that sedges such as *Eriophorum* spp. provide fresh substrate through their roots, which is then converted into methane (Ström et al., 2005).

Previously, it has been shown that methane emissions are lower in areas that have a *Sphagnum* cover (Hines et al., 2008) and this has mostly been contributed to the low coverage of vascular plants in these areas, limiting plant transport of methane from the anoxic zone to the atmosphere. However, apart from the lower plant transport, it is also likely that oxidation of methane is higher in *Sphagnum* (Vecherskaya et al., 1993; Sundh et al., 1995). Raghoebarsing et al. (2005) have shown that this is caused by methanotrophic bacteria, which live inside or on submerged *Sphagnum*, with especially high methane oxidation rates in submerged *Sphagnum cuspidatum*. Through photosynthesis, the plant provides oxygen to these bacteria which is used to oxidize methane below the water table, in turn providing CO_2 for photosynthesis back to *Sphagnum*. Raghoebarsing et al. (2005) also estimate that, due to this added CO_2, this cooperation increases the carbon uptake of *Sphagnum* by 5 to 20%. This system could thus explain the high carbon burial found in peatlands.

Recently, it has been shown that these methanotrophic endophytes are very common around the world (Kip et al., 2010), with varying rates of oxidation. However, there are very few studies that relate field observations of methane emissions from submerged *Sphagnum* to this specific type of bacteria since most studies on the spatial variations of methane fluxes focus on water level, NPP, vascular plant cover or oxidation in the aerated part of *Sphagnum* (Fechner and Hemond, 1992; Bubier, 1995; Frenzel and Rudolph, 1998; Greenup et al., 2000; Joabsson and Christensen, 2001; Christensen et al., 2003; Kutzbach et al., 2004; Basiliko et al., 2004; Ström et al., 2005; Minkkinen and Laine, 2006; Hines et al., 2008), while oxidation in inundated areas with *Sphagnum* vegetation is much less studied (Kip et al., 2010; Larmola et al., 2010). In this paper, we compare two inundated vegetation types in Northeastern Siberian tundra and show

BGD
7, 8521–8551, 2010

that methane emissions are significantly lower in a submerged *Sphagnum*/sedge vegetation type, in comparison to sedge vegetation with no *Sphagnum* presence, and investigate how methanotrophic bacteria in submerged *Sphagnum* attribute to this difference.

2 Materials and methods

2.1 Study site

The study site is located in the nature reserve "Kytalyk" in Northeastern Siberia (70°49′44.9″ N, 147°29′39.4″ E), about 30 km NW from the town of Chokurdakh in the Sakha Republic (Yakutia), Russian Federation (as shown in Fig. 1). It is situated in a former thermokarst lake that drained in the past when it was intersected to the south by the Berelekekh (Yelon) river, a tributary to the Indigirka river. The climate is cold and continental with an average annual temperature of −10.5 °C and extremes as low as −25 to −45 °C in winter and 5 to 25 °C in summer. Snowmelt usually occurs at the start of June and half way through September temperatures start to drop below zero again. While most snow is gone in mid June, bud break does not occur before the end of June or early July, together with the first warm days of the year. This limits the growing season to the months of July and August.

Summer temperatures are highly variable due to the large contrast between winds from the North and South. Northern winds blow cold air from the East Siberian sea (approx. 100 km away) while Southern winds bring hot summer air from the Siberian interior. This dependency on wind direction also means that the air temperature can drop by as much as 20 °C in just two days if the wind direction changes from South to North. Since methane emissions are sensitive to temperature change, this also has an obvious effect on emissions on the short-term.

Annual precipitation is about 200 to 250 mm with approximately half of it falling as rain during the growing season. The other half falls in the rest of the year, mostly as

BGD
7, 8521–8551, 2010

snow. Although this amount of precipitation is similar to the yearly total in semi-arid areas, total evaporation is much lower and thus the soil remains very wet and plenty of water is available for plant growth.

The vegetation is classified as graminoid tundra (tussock-sedge, dwarf shrub, moss tundra, cf. circumpolar arctic vegetation map, Walker et al., 2005). The spatial heterogeneity of the vegetation is related to the presence of ice-wedge polygon microtopography leading to differences in soil water saturation. The higher and drier parts are dominated by either *Betula nana* and *Salix pulchra* dwarf shrubs with mosses or *Eriophorum vaginatum* hummocks interspersed with *Salix pulchra* dwarf shrubs and mosses. Towards the center of a polygon soil conditions get wetter; *Betula nana* is no longer present and *Salix pulchra* cover gets more sparse while *Sphagnum* spp. cover increases and *Carex aquatilis* and *Eriophorum angustifolium* appear. Dominant *Sphagnum* species include *S. balticum*, *S. compactum*, *S. subsecundum* and *S. squarrosum*. The center, lowest part of polygons are usually inundated and vegetation is dominated by *Carex aquatilis* and *Eriophorum angustifolium* while *Sphagnum* spp. cover is largely reduced or absent. Similar vegetation exists at the edges of ponds that are created by melting ice wedges, although transitions can be more abrupt. A more elaborate site description and comparison with other sites has previously been given by van Huissteden et al. (2005) and van der Molen et al. (2007).

2.2 Methane flux measurements

To find areas that were suitable for comparison, the vegetation at each measurement location was classified according to vegetation, geomorphology and water availability as described by van Huissteden et al. (2005). This classification identifies 12 different classes. Of these 12, the vegetation types TW1 and TW4, as described in Table 1, were used to compare between plots with and without *Sphagnum*. Both these vegetation types are usually inundated, which means there is no aerated part of the soil or *Sphagnum* layer. TW1 is a vegetation type dominated by *Eriophorum angustifolium* and *Carex aquatilis* (typical cover of 40 to 95%), where *Sphagnum* is mostly absent.

BGD
7, 8521–8551, 2010

TW4 is a vegetation type that is dominated by *Sphagnum* (cover of 50 to 100%) but a substantial amount of vascular plants such as *Carex aquatilis*, *Eriophorum angustifolium* and *Comarum palustre* remain (typical cover of 20 to 30%). This difference in vascular plant cover is possibly due to competition between *Sphagnum* and vascular plants, resulting in a lower cover of the latter (Heijmans et al., 2002).

The other ten classes were not considered since they either referred to the floodplain (where no *Sphagnum* is present and conditions are completely different from the tundra terrace), to dry vegetation types with a water table below the surface or to areas with *Sphagnum* where the water table was below the top of the *Sphagnum*.

Measurements were performed on 5 plots of the TW1 vegetation type and 4 plots of the TW4 vegetation type, for which the spatial variation of vegetation within each class was taken into account visually. The plots were located in close vicinity to each other, often only separated by a few meters. Furthermore, only those measurement days were selected where both vegetation types were inundated, to avoid differences in the measured fluxes due to oxidation of methane in the aerated part of the soil or *Sphagnum* layer.

Chamber flux measurements were performed in the summer of 2007 between 18 July and 6 August with the use of an INNOVA 1412 Photoacoustic field Gas-monitor (LumaSense Technologies A/S, Ballerup, Denmark), following the same measuring practice as described in van Huissteden et al. (2005). Each day the same 9 plots were measured, to avoid the occurrence of variations in the measurements due to spatial differences. For each measurement point, a plastic collar of 30 cm in diameter and 10 cm in height was placed carefully in the top soil with a 14 dm^3 dark plastic chamber on top. A water lock was used in between the chamber and collar to prevent gas leakage. For 8 min, methane concentrations were measured 5 times with a 2-min interval. The measurement was kept this short to make sure that the air in the chamber would not warm up too much and this was further monitored with the use of a small thermometer inside the chamber. Also, the sampled air was first passed through a tube containing soda lime and a silica gel, which removed CO_2 and reduced water

BGD
7, 8521–8551, 2010

vapor concentrations, to prevent cross-interference at high concentrations.

Fluxes were determined by linear interpolation of the measurements, accounting for air temperature, air pressure and, if there was standing water above the surface, also for reduced air volume in the chamber. Quality control was done by calculating the root mean square error (RMSE) of the linear regression. A high RMSE would occur in the case of a non-linear increase of concentration, in which case the measurement would have to be rejected, although this did not occur for the studied period and vegetation types. In some cases the total change in concentration would be very low (<1 ppm) and erratic in behavior, due to the measurement accuracy of the device (0.4 ppm). In these cases fluxes could not be determined accurately and because the possibility of some leakage cannot be excluded fully (although unlikely), these measurements were excluded to avoid biased means. Together with each flux measurement, the water table level, thickness of the active layer and soil temperatures at 0, 10 and 20 cm were also measured.

2.3 Microbiological analysis

In July 2008, samples of submerged *Sphagnum* were collected from two sites, NS1 and NS2, with a similar vegetation distribution as the TW4 flux sites. These were brought back to the Netherlands for microbiological analysis. The samples were transported in closed plastic bags and kept cool as much as possible. During transits by airplane they were no longer than 20 h without active cooling. Incubation experiments were performed in September 2008.

Potential methane-oxidizing activity was measured by incubating whole *Sphagnum* plants, 20 grams of moist mass, in a 120 ml serum bottle sealed with airtight grey butyl rubber stoppers and aluminum caps. Before incubation, the *Sphagnum* plants were thoroughly washed 3 times with sterile demineralized water. 1 ml of methane (100% pure, Air Liquide, the Netherlands) was added to each flask and the methane concentration in the flasks was measured on a HP 5890 gas chromatograph equipped with a flame ionization detector and a Porapak Q column (100/120 mesh). The methane

BGD
7, 8521–8551, 2010

oxidizing activity test was performed in triplicate on ice and at 4, 10 and 20 °C in the dark. Following the incubations, the *Sphagnum* mosses were dried in a vacuum stove at 70 °C to determine the dry weight.

Oxidation rates were determined at three separate stages. The initial methane oxidation rate, rate 1, is measured between 0 and 18.5 h, the second methane oxidation rate is determined between 20 and 46 h, after adding new methane, and the last methane oxidation rate, rate 3, is determined after 46 h when all the samples were incubated on ice. Since no peat bog water sample was available, the first wash water served as a control. Methane concentrations in the bottles were measured every hour or every day, depending on the activity, while methane oxidation rates were determined by regression analysis of the data points that show a linear methane oxidation.

Since oxidation rates are determined as $\mu mol\, CH_4\, gDW^{-1}\, day^{-1}$, the amount of dry weight of *Sphagnum* per square meter was needed to compare these rates with methane emissions in the field. Therefore, four 0.25 m^2 plots, with similar vegetation to the observed methane plots, were selected and all *Sphagnum* was collected. This was subsequently dried in an oven for a week at 60 °C and weighed afterwards. The obtained weight could then be multiplied with the oxidation rates to give an oxidation rate per square meter.

2.4 Flux modeling

CH_4 fluxes from the Kytalyk site have been modeled by Petrescu et al. (2008) and van Huissteden et al. (2009) using the PEATLAND-VU model that includes a version of the Walter and Heimann (2000) wetland CH_4 flux model (van Huissteden et al., 2006). Model sensitivity analysis has been performed using the GLUE method (General Likelihood Uncertainty Analysis, e.g. Beven, 2008) by van Huissteden et al. (2009). In this method, a large number of model runs are performed with randomly selected values for the studied parameters. The results of each run are compared with measurement data and an objective function is calculated that indicates the model fit. Here 1000 runs were used, which were compared with the measurement data for TW1 and TW4.

BGD
7, 8521–8551, 2010

The distribution of objective function values vs. parameter values shows how sensitive the model performance is to variations in the parameter set. We assume that a distinct clustering of high objective function values within a certain range of parameter values represents an approximation of the true values, given the model structure. We used the Nash-Sutcliffe efficiency for grouped site data (Nash and Sutcliffe, 1970), which compares the model results with site group average and standard deviation. The GLUE analysis has been compared for both vegetation types. We focussed on parameters that determine oxidation of CH_4 during plant transport and plant transport rate (van Huissteden et al., 2009):

- f_{ox}: within plant oxidation factor (0–1), reducing the amount of emitted CH_4 from plants.
- V_{transp}: plant transport factor (0–15), increasing the amount of emitted CH_4 from plants.
- f_{shoots}: fraction (0–1) of net primary production (NPP) allocated to aboveground shoots.
- Z_{roots}: maximum root depth (0.1–0.6 m).
- P_{max}: maximum daily NPP (0.001–0.005 $kgC\,m^2\,day^{-1}$).

3 Results

3.1 Methane flux measurements

Figure 2 shows the measured fluxes, while Fig. 3 shows the water level, active layer thickness and temperature for the two vegetation classes. For each day, the fluxes of the class with, and without submerged *Sphagnum* have been plotted next to each other and the error bars represent the standard deviations of the measurements. Average daily fluxes ranged from 3.6 to 12.3 $mg\,CH_4\,m^{-2}\,hr^{-1}$ for TW1 and

BGD
7, 8521–8551, 2010

from 0.7 to 7.8 mg CH_4 m^{-2} hr^{-1} for TW4. The averages of the measured fluxes were 8.0 ± 4.7 mg CH_4 m^{-2} hr^{-1} and 4.1 ± 3.1 mg CH_4 m^{-2} hr^{-1} for TW1 and TW4, respectively. From the difference of the means it follows that the emissions from the vegetation type with submerged *Sphagnum* were half as much as emissions from the vegetation type without *Sphagnum*.

For each measured day, the plots without *Sphagnum* show higher fluxes than the plots with *Sphagnum*, as expected. However, since the measurements were performed manually, only a limited amount of measurements (4 to 5) could be done per vegetation class per day. This led to a high standard deviation and there is some overlap between the two vegetation types. To show statistically that the two vegetation types do show different fluxes, a linear mixed model (Type III test of fixed effects with an AR(1) covariance structure, e.g. Littell et al., 1998) was performed with the use of PASW Statistics 18.0 (SPSS Inc., Chicago, IL). This method was preferred over a repeated measures ANOVA since the latter cannot handle missing data. The mixed model showed that the two vegetation types are indeed different at a 95% significancy level ($p = 0.046$).

3.2 Microbiological analysis

High methane oxidation rates were found for the samples NS1 and NS2 at 4 °C, 10 °C and 20 °C, as shown in Table 2. Methane oxidation rates varied between 32 and 80 µmol CH_4 gDW^{-1} day^{-1} and addition of new methane did not result in increased rates. Surprisingly, all samples showed activity between 2.7 and 7 µmol CH_4 gDW^{-1} day^{-1}, when incubations were continued on ice. Methane oxidation rates measured in the water controls were negligible since rates were 0.04 ± 0.02 µmol CH_4 gDW^{-1} day^{-1} on average. A more in-depth microbiological analysis on the bacterial community of these samples has been published previously by Kip et al. (2010).

BGD
7, 8521–8551, 2010

The amount of dry weight of *Sphagnum* per m^2 was determined to be 415 ± 250 g, which means that an oxidation rate of 10 µmol CH_4 gDW^{-1} day^{-1} equals 2.8 ± 1.7 mg CH_4 $m^{-2}hr^{-1}$. The observed temperature range in the field was roughly between 4 and 12 °C and, according to Table 2, oxidation rates at these temperatures vary between 30 and 40 µmol CH_4 gDW^{-1} day^{-1} or 8.4 ± 5.0 to 11.1 ± 6.6 mg CH_4 m^{-2} hr^{-1}, which is about a factor of two larger than the observed difference between the two vegetation types.

3.3 Flux modeling

The results of the model runs for the two vegetation types are shown in Fig. 4. For most days the model agrees quite well and the modeled fluxes fall within the standard deviations of the observed values. Poor model performance only occurs on 18 July, when observed fluxes of sites without *Sphagnum*, TW1, are clearly higher than those modeled and on 31 July, when observed fluxes of both vegetation types are much lower than those modeled.

In Fig. 5, the results of the GLUE analysis are shown for all runs where the Nash-Sutcliffe efficiency was larger than zero, and for the two parameters that are expected to influence methane emission most strongly, f_{ox} and V_{transp}. Of the 1000 random model runs, fewer runs gave a positive Nash-Sutcliffe efficiency for sites with *Sphagnum* than without *Sphagnum* and therefore fewer points are plotted for that vegetation type. The significance threshold ($p < 0.1$, NS = 0.551 according to F test) was not crossed, although this is common for methane emission modelling (van Huissteden et al., 2009); for TW1 the threshold is approached. More important is, that the the GLUE results show a strong identifiability of parameter f_{ox} and a clear difference in average parameter values for TW1 and TW4 (resp. 0.62 and 0.86, the latter value resulting in significantly higher oxidation; tested with t-test, $p < 0.001$). For the other parameters, no significant difference can be detected, in particular not for V_{transp} . Given the model structure, which includes modelling of plant transport and oxidation rate, it is likely that

BGD
7, 8521–8551, 2010

a difference in parameter identifiability and parameter values indicate realistic factors that influence the methane fluxes. This indicates that the difference in fluxes between the two vegetation types is best explained by differences in oxidation, and not by the other parameters tested.

4 Discussion

The measurements of the methane emissions from the two inundated vegetation types show that fluxes from vegetation without submerged *Sphagnum*, TW1, was $8.0 \pm 4.7\,\mathrm{mg\,CH_4\,m^{-2}\,hr^{-1}}$ and $4.1 \pm 3.1\,\mathrm{mg\,CH_4\,m^{-2}\,hr^{-1}}$ for vegetation with submerged *Sphagnum*, TW4; a difference of a factor of two. The standard deviation on the averages is quite large since the measured plots were selected in such a way that they represent the spatial variation for that vegetation type and large variations between individual measurements are therefore to be expected. The linear mixed model showed that the two vegetation classes do have different fluxes at a 95% significancy level ($p = 0.046$). In previous years, while using a roving manner for measuring fluxes at the same site, a similar difference between these two vegetation types was observed (van Huissteden et al., 2005, 2009, van der Molen et al., 2007), which confirms these results. Furthermore, others have observed the high reduction in fluxes between these two types of vegetation as well, such as Hines et al. (2008), who similarly reported a 50% lower flux in a mixed vegetation of sedges and *Sphagnum*.

The two vegetation types observed also showed a large differences in vascular plant cover. Since these plants are very important to the emission of methane in wetlands (Joabsson et al., 1999), it would have been preferable to select sites where vascular plant cover was similar and only *Sphagnum* cover would be different. However, in the field it became clear that vascular plant cover was always higher for vegetation without *Sphagnum*, TW1, than for vegetation with *Sphagnum*, TW4. This could be due to competition between *Sphagnum* and vascular plants, as has been shown by Heijmans et al. (2002), making this difference a de facto situation that cannot be avoided.

BGD
7, 8521–8551, 2010

Even so, the question remains whether the observed difference in fluxes can be attributed to vascular plant cover alone. Therefore, the average methane flux of all measurement sites is plot against vascular plant cover, obtained from a vegetation mapping for each site, in Fig. 6. As expected, the TW4 sites show lower fluxes and have a lower vascular plant cover than TW1. However, this does not necessarily mean that this is due to a relationship with vascular plant cover. The difference in fluxes from the two classes could alternatively be explained by plotting them along *Sphagnum* cover, since TW4 has a high *Sphagnum* cover and TW1 none. Therefore, it is preferable to study relationships between vascular plant cover and fluxes within the vegetation classes themselves. In that case, Fig. 6 clearly shows that there is no pattern with vascular plant cover and no significant regression could be found. Actually, the second highest fluxes in the TW1 class were measured in a plot with a vascular plant cover close to that of the TW4 plots.

Furthermore, this lack of a significant relationship with vascular plant cover is supported by the modeling study. Here, it was clearly shown that oxidation rate best explained the difference in observed fluxes, while plant transport proved to be a poor predictor to the magnitude of the fluxes. A good model result was only observed if oxidation rates were set 50% higher in areas with *Sphagnum* compared to the areas without *Sphagnum*, while no statistical difference in model performance could be found by varying the plant transport parameter. So, although the two vegetation types have different vascular plant cover percentages, the measurements and model show that vascular plant cover explains the differences observed in the fluxes poorly.

When compared to previous research on plant transport (Joabsson et al., 1999; Tsuyuzaki et al., 2001; Ström et al., 2003; Christensen et al., 2003; Kutzbach et al., 2004), this result seems counter intuitive, but it can be explained by the fact that vascular plants usually increase methane fluxes by bypassing the aerated parts of the soil, where methane would otherwise be oxidized. Since the studied vegetation types were always inundated, there was no aerated soil layer with associated methane oxidation. Therefore, the difference between net transport to the atmosphere through

BGD
7, 8521–8551, 2010

aerenchyma and upward diffusion is much smaller and the relative contribution of plant transport to net methane emissions is reduced.

While the measurements and model do not point to vascular plant cover as a predictive factor of the observed differences, the incubation study found very high oxidation rates of methane in submerged *Sphagnum*. This confirms the outcome from the model that oxidation is a good predictor to the observed differences. When these rates are converted to mg CH_4 m^{-2} hr^{-1}, with the use of the amount of dry weight of Sphagnum per m^2, the lab analysis showed potential rates that were larger than the difference in fluxes between the two vegetation types. Although high, these rates are not atypical when compared to oxidation rates for incubations of submerged *Sphagnum* samples from around the world. For example, a site from Argentina showed similar oxidation rates at 20 °C (Kip et al., 2010). Although, most surprisingly, the samples from the studied site still showed some methane oxidation at 4 °C and on ice. No methane oxidation could be measured under those circumstances for the other samples incubated by Kip et al. (2010), who also showed, with the use of a pmoA-based microarray and enrichment cultures, that this behavior is attributed to an unique methanotrophic bacterial community present in the Northeastern Siberian ecosystem. Apparently, this community is active over a very large temperature range, 0–20 °C, which explains why oxidation rates stay high in the cold Siberian soil.

Although these incubations are difficult to translate to quantitive oxidation rates in the field, they do indicate that there is a very high potential for oxidation of methane in submerged *Sphagnum*. This makes it, in the context of the measurements and the model, very likely that these bacteria play a large role in the recycling of methane at the studied site, explaining the observed differences to a great deal, if not all.

BGD
7, 8521–8551, 2010

5 Conclusions

In this study, we compared two inundated vegetation types and found methane emissions from areas dominated by submerged *Sphagnum*, with some sedges, to be two times lower than the emissions from inundated vegetation dominated by sedges, but without *Sphagnum*. Although previous research has shown that these differences could be attributed to a difference in vascular plant cover, which increases plant transport (Joabsson et al., 1999; Tsuyuzaki et al., 2001; Ström et al., 2003; Christensen et al., 2003; Kutzbach et al., 2004), this study could not find such a relationship. A possible explanation could be that usually these plants provide a pathway for methane to bypass the aerated part of the soil, thus preventing oxidation. In this study, only inundated vegetation types were examined and therefore no aerated part of the soil needed to be bypassed. The relative contribution of plant transport to the total emission of methane would therefore be reduced.

A more likely explanation to the observed differences was provided by an incubation study of submerged *Sphagnum*, where samples from the studied site showed very high oxidation rates of methane, even at 4 °C and on ice, up to two times as high as the difference in fluxes in the field. This suggests that below the water table oxidation in submerged *Sphagnum* is highly important to explaining the differences in methane emissions from these inundated vegetation types. To further support these findings, the fluxes were modeled with a process model, which also showed that oxidation, not plant transport, was the distinctive factor that explained the observed differences. This emphasizes the importance of these methanotrophic endophytes found in submerged *Sphagnum*, while the effect of plant transport on the observed fluxes in the inundated vegetation types of this study is less pronounced.

We conclude, by combining flux chamber measurements, microbial analysis and modeling, that this type of methanotrophic bacteria, that lives in a cooperation with submerged *Sphagnum*, is an important factor in the recycling of methane in the soils of inundated areas in tundra. On average, emissions were two times lower in a submerged

BGD
7, 8521–8551, 2010

Sphagnum/sedges mixed type of vegetation, compared to a submerged vegetation type without *Sphagnum* and dominated by sedges. This difference is most likely due to, for a large part, oxidation of methane within submerged *Sphagnum*.

Respective surface cover of the two vegetation types is only 5.4% and 13.3% at the studied site (van der Molen et al., 2007), but almost all emitted methane comes from these areas. This means that the vegetation type dominated by submerged *Sphagnum*, represents 30% of the methane emitting surface. If we assume a ratio of 2:1 in the emissions between the two vegetation types, it can be estimated that oxidation by methanotrophic endophytes plays a large role in 15% of the upscaled net methane emission from this tundra site. Also, since methane is converted into CO_2 by these bacteria and made available for *Sphagnum* to be used in photosynthesis and converted into plant tissue (Raghoebarsing et al., 2005; Kip et al., 2010), this cooperation promotes carbon storage in wetlands as well.

Climate models predict a high temperature increase in the arctic in the near future (Zwiers, 2002; Chapin III et al., 2005; Lawrence et al., 2008), which would also lead to higher methane oxidation rates by these methanotrophs (Kip et al., 2010). However, it is suggested that an increase in temperature could also have a negative impact on the spread of *Sphagnum* (Gunnarsson et al., 2004; Heijmans et al., 2008) in favor of an increase in vascular plant cover (Epstein et al., 2004). The areal spread of *Sphagnum* in the inundated parts of tundra and the contribution of these methanotrophic bacteria to methane recycling and carbon storage in soils, in relation to their response to a warmer climate, could therefore be highly important in predicting future methane emissions in the Arctic.

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BGD
7, 8521–8551, 2010

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BGD
7, 8521–8551, 2010

Table 1. Description of the studied vegetation classes.

Code	Site class	Soil	Water table	Vegetation	Vascular plant cover
TW1	depression, diffuse drainage	organic on mineral	0–15 cm	*Eriophorum angustifolium*, *Carex aquatilis*	40% to 90%
TW4	low polygon centre, other depressions	organic	0–10 cm	*Sphagnum*, *Carex aquatilis*, *Comarum palustre*	20% to 30%

BGD
7, 8521–8551, 2010

Table 2. Mean methane oxidation rates for the two analyzed *Sphagnum* samples from sites NS1 and NS2 at different incubation temperatures. The values are in µmol CH_4 g^{-1} dry weight/day ± standard deviation ($n = 3$).

Temperature (°C)	4°	10°	20°
		NS1	
Oxidation rate1	40 ± 0.9	58 ± 0.5	80 ± 0.3
Oxidation rate2	30 ± 0.7	42 ± 0.4	75 ± 0.6
		NS2	
Oxidation rate1	33 ± 0.3	39 ± 0.4	54 ± 0.2
Oxidation rate2	32 ± 0.6	38 ± 0.5	62 ± 0.5

BGD
7, 8521–8551, 2010

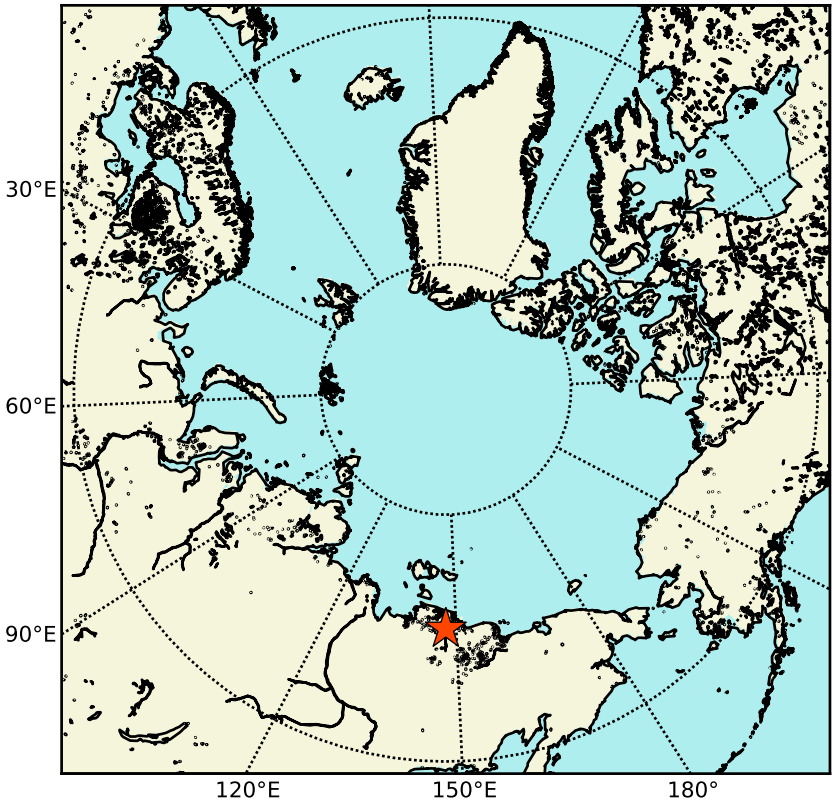

Fig. 1. Location of the research site within Northeastern Siberia.

BGD
7, 8521–8551, 2010

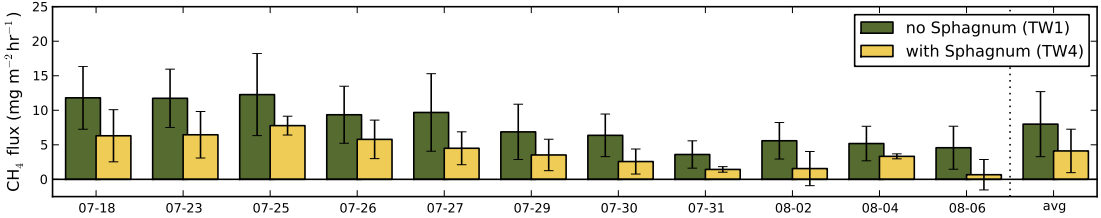

Fig. 2. Daily fluxes for terrain types without (TW1) and with *Sphagnum* (TW4). Measurements are shown per day and on the right the average flux of the two vegetation types is shown. Error bars denote the standard deviation of the measurements.

BGD
7, 8521–8551, 2010

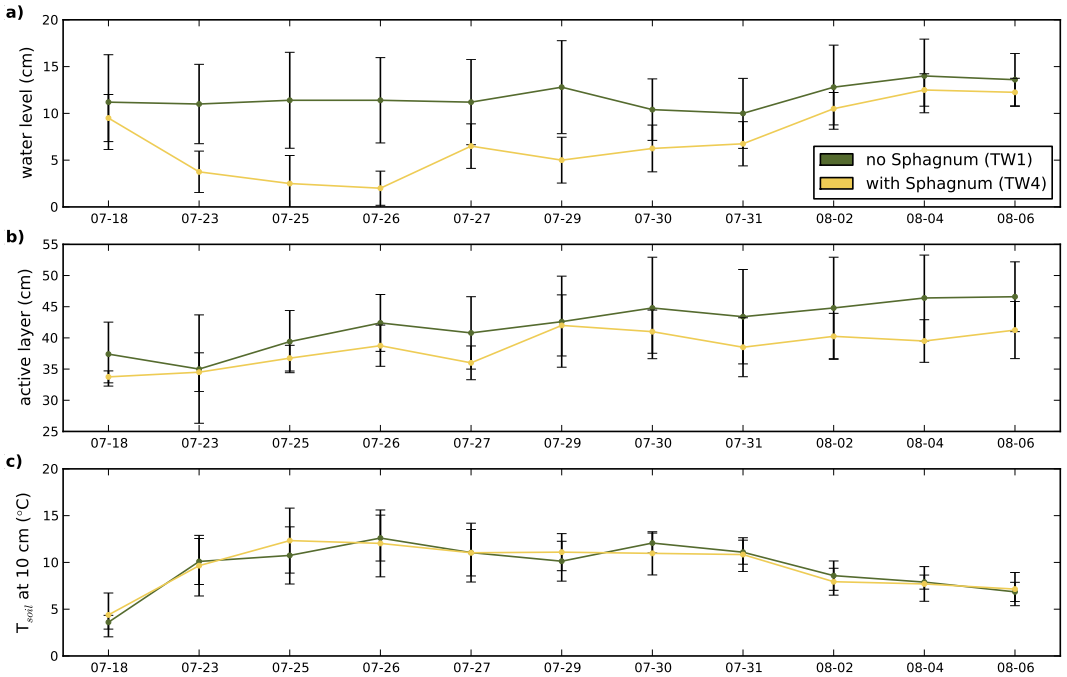

Fig. 3. Environmental parameters during the measurement campaign for the two vegetation types. Dark grey shows the data for the vegetation type without *Sphagnum*, TW1, while the data for the vegetation type with *Sphagnum*, TW4, is shown in light grey. Error bars denote standard deviations. **(a)** Daily water level above the surface. **(b)** Active layer thickness. **(c)** Daily soil temperature at 10 cm depth.

BGD
7, 8521–8551, 2010

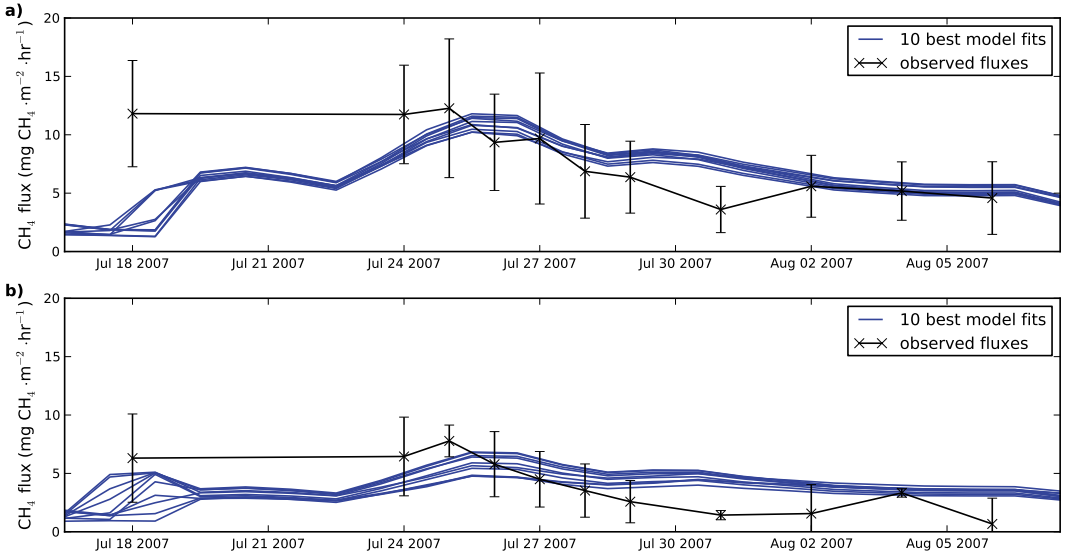

Fig. 4. The 10 best model fits (grey) plotted together with observed data (black). Error bars denote standard deviations.

BGD
7, 8521–8551, 2010

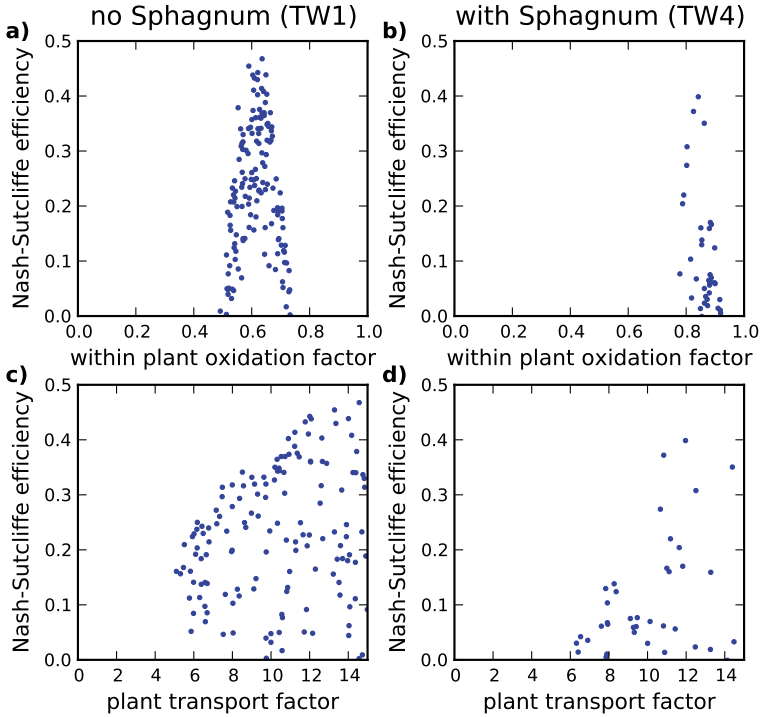

Fig. 5. GLUE analysis of model parameters for both vegetation types. On the left side (**a** and **c**) the results for the vegetation type without *Sphagnum*, TW1, are shown, while the results for the vegetation type with *Sphagnum*, TW4, are shown on the right hand side (**b** and **d**). The top row (a and b) shows the Nash-Sutcliffe efficiency for within plant oxidation of methane, f_{ox}, for both vegetation types, while the bottom row (c and d) shows the Nash-Sutcliffe efficiency for the plant transport factor, V_{transp}.

BGD
7, 8521–8551, 2010

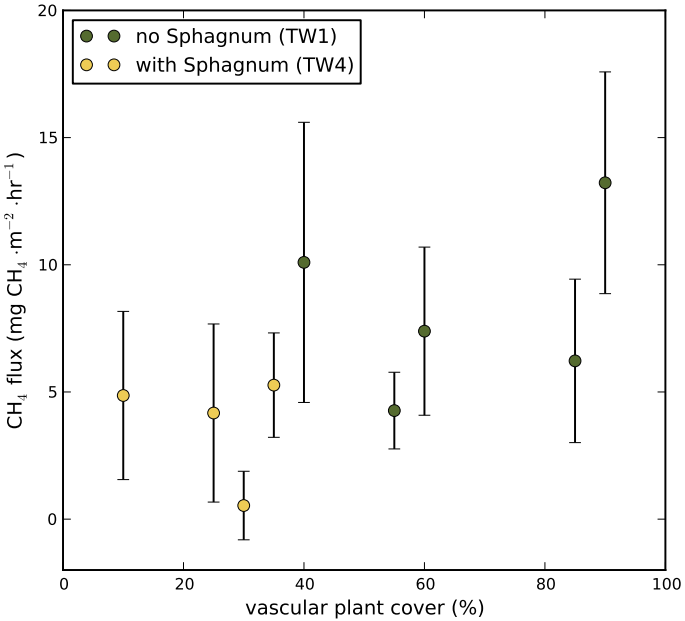

Fig. 6. Average methane fluxes of each measurement site, plot along its vascular plant cover. The error bars denote standard errors along all measurement days. Although there is a difference in vascular plant cover and methane flux between the two vegetation types TW1 and TW4, within each vegetation class there is no significant increase with vascular plant cover.

BGD
7, 8521–8551, 2010